CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Picloram, Potassium Salt

Chemical Code # 1099, 5330, Tolerance # 292, 52424

Original: 7/30/86
Revised: 12/2/87, 3/7/88, 6/22/99, and 10/22/99

I. DATA GAP STATUS

Chronic toxicity, rat:.	No data gap; no adverse effect
Chronic toxicity, dog:	No data gap; no adverse effect
Oncogenicity, rat:	No data gap; possible adverse effect indicated[1]
Oncogenicity, mouse:	No data gap; no adverse effect
Reproduction, rat:	No data gap; no adverse effect
Teratology, rat:	No data gap; no adverse effect
Teratology, rabbit:	No data gap; no adverse effect
Gene mutation:	No data gap; no adverse effect
Chromosome effects:	No data gap; possible adverse effect indicated
DNA damage:	No data gap; no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 170554 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T991022
Revised by Thomas Moore, 10/22/99

Picloram (CC593), triisopropanolamine salt (CC 1099) and the potassium salt (CC 5330) are grouped for toxicological testing.

[1] Neoplastic nodules in the liver were observed in an older study with Osborne-Mendel rats, but not in two recent studies with Fischer 344 rats.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

52424-006; 161904; "Picloram: Two-Year Dietary Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats"; (P.F. Cosse et. al., Health & Environmental Sciences, Dow Chemical Co., Midland, MI, Study ID # K-038323-056, 12/22/92). Picloram (AGR 0274601, 81.8% a.i.) was administered in test diets to 50 Fischer 344 rats/sex/dose at 0, 250, or 500 mg/kg/day for two years. 10 additional rats/sex/dose were used for interim sacrifice at 12 months. No treatment-related effects in mortality, body weight, food consumption, ophthalmology, hematology, and functional observation battery observations were reported. 1/10 male from the 250 mg/kg group and 10/10 males and 3/8 females from the 500 mg/kg group that had blood in urine also exhibited histological findings consistent with chronic progressive glomerulonephropathy. Increased blood urea nitrogen (BUN) was reported in males treated at 500 mg/kg. Mean absolute liver weights from females in the 250 mg/kg group and mean absolute and relative liver weights from females in the 500 mg/kg group were noted without any signs of abnormal liver histology at 2 years. Histopathological exam of rats at the 1 year interim sacrifice revealed slight increased size of centrilobular hepatocytes accompanied by altered tinctorial properties (increased eosinophilia) in males (2/10, 7/10, and 10/10 from 0, 250, and 500 mg/kg groups, respectively). This type of liver effect was not detected in females or in any rats at terminal sacrifice at 2 years. Unilateral or bilateral renal papillary necrosis was reported in 10/50 males dosed 500 mg/kg/day. **No adverse effects.** NOEL (M/F) < 250 mg/kg/day (based on chronic progressive glomerulonephropathy). **Supplemental** (Leung, 8/12/98).

52424-004, 161898; 292-015 to -019, 41609 - 41613 " Picloram: A Two-year Dietary Chronic Toxicity Oncogenicity Study in Fischer 344 Rats" (T.D. Landry et. al., Health & Environmental Sciences, Dow Chemical USA, Midland, MI, 1/9/86) Picloram (93%, lot # AGR-177446 with hexachlorobenzene at 197 ppm by LC) administered at 0, 20, 60, or 200 mg/kg/day in the diet for 2 years to 70 Fischer 344 rats/sex/group. 10 rats/sex/dose were sacrificed at 6 and 12 months (record #2851). NOEL (M/F) = 20 mg/kg/day; Effects on liver at 60 and 200 mg/Kg were described as increased size of centrilobular hepatocytes and altered tinctorial properties (males: 11/50 and 20/50 vs. 2/50, respectively, $p < 0.05$; females: 8/50 and 10/50 vs. 2/50, respectively, $p < 0.05$) with increased organ weight at high dose. Male rats receiving 60 or 200 mg/kg had a greater degree of pancreatic acinar tissue atrophy than controls (7/50 and 6/50 vs. 1/50, respectively, $p < 0.05$). However, there was no effect on the total number of rats with some degree of acinar atrophy. No oncogenic effect was reported. Increased pigment in the proximal convoluted tubule noted in kidneys from males treated at 200 mg/kg at 12 months not seen at 2-year sacrifice. Due to lack of ophthalmological examination, the status of this study was changed to **Unacceptable and not upgradeable**. **No Adverse effects indicated.** (Gee, 6/3/86; updated and revised, Leung, 7/27/98).

CHRONIC TOXICITY, RAT

See Combined, Rat.

CHRONIC TOXICITY, DOG

** 52424-004; 161899; "Picloram: 12-Month Dog Chronic Dietary Toxicity Study"; (T. Barna-Lloyd et. al., Health & Environmental Sciences - Texas, Lake Jackson Research Center, Freeport, TX, Study ID # K-038323-040, 6/8/88). Picloram (technical grade, AGR-219562, 93.9% purity) was fed in diets to beagle dogs (4/sex/dose) at 0, 7, 35, or 175 mg/kg/day for 1 year. One mid dose female died on day 67. Histopathological and hematological findings suggested that the underlying cause of death was an immune-mediated hemolysis, with resultant anemia and tissue changes secondary to hypoxia. Increased absolute liver weight in high dose males and relative liver weight in high dose animals were noted. No histopathological lesions or abnormal findings associated with the increased liver size and weight were detected. **No adverse effects**. NOEL (M/F) = 35 mg/kg/day (increased relative liver weight). **Acceptable**. (Leung, 8/5/98).

ONCOGENICITY, RAT

292-010; 36437; "Bioassay of Picloram for Possible Carcinogenicity (Administered in Feed to Rats-80 Weeks)" (M. Steinberg et. al, NCI, 1978) Picloram (>90%) fed in the diet at 10,000 or 20,000 ppm for 39 weeks and 5000 or 10,000 ppm for 41 weeks and observed for 33 weeks --113 weeks total; 10/sex for controls, 50/sex/group for low and high doses; **positive for treatment-related liver onco** in high dose females, questionable response in livers of other group with neoplastic nodules in 0/10 controls, 5/50 in low dose and 7/49 in high dose females; focal cellular changes in liver of males: 0/10 controls, 12/49 low and 5/49 high dose, females: 1/10 control, 8/50 low and 18/49 high dose. In thyroids, hyperplasia and adenomas were found in treated groups but not controls. Without historical data and with such small concurrent controls, the meaning of these findings is made difficult. **Unacceptable** (no individual data, drastic change in dose levels at week 40 of study, too few controls, limited dosing for 80 weeks followed by control diet for 33 weeks, 2 dose levels only, incomplete histopathology), not upgradeable. EPA one-liner: Rated as weakly positive in females for onco effect at high dose without a grade notation (10/26/84). (Gee, 11-21-85; updated, Leung, 7/28/98).

ONCOGENICITY, MOUSE

** 52424-007; 161905; "Picloram: Two-year Dietary Oncogenicity Study in B6C3F1 Mice" (W.T. Stott et. al., Health & Environmental Sciences, Dow Chemical Co., Midland, MI, Study ID # K-038323-058, 12/24/92). Picloram (AGR # 274601, 82% purity) was administered in diets to 50 mice/sex/ dose at 0, 100, 500, or 1000 mg/kg/day for 2 years. 10 additional mice/sex/dose were used for interim sacrifice. No treatment-related effects on clinical signs, survival rates, body weights, food consumption, ophthalmology, hematology, gross necropsy and histopathology were detected. Marginal increased (106 to 107% of control, $p < 0.05$) kidney weights of high dose males after 24 months of dosing was not associated with any histological changes in renal tissues. Therefore, this effect was not considered to toxicologically significant. **No adverse effects**. NOEL (M/F) = 1000 mg/kg/day (no effects detected at HDT). **Acceptable** (Leung, 8/19/98).

292-010; 36436; "Bioassay of Picloram for possible Carcinogenicity (Administered in Feed to Mice-80 Weeks)" (M. Steinberg et. al., NCI, 1978) Picloram (>90%) fed in the diet at 5000 or 10,000 ppm for 1 week, 2500 or 5000 ppm for weeks 2-80 followed by 10 weeks of observations; 10/sex for controls, 50/sex/group for low and high dose levels; **no oncogenic effect** reported; **Unacceptable** (no individual data, too few controls, only 2 dose levels, marked change in dose levels at week 1, incomplete histopathology, no evidence that MTD achieved, no clinical obs), not upgradeable. EPA one-liner: Negative with no grade notation (10/26/84). (Gee, 11-21-85; updated, Leung, 7/28/98).

REPRODUCTION, RAT

** 52424-008; 161906; "Picloram: Two Generation Dietary Reproduction Study in Sprague-Dawley Rats" (W. J. Breslin et. al., Health & Environmental Sciences, Dow Chemical Co., Midland, MI, Study ID # K-038323-057, 10/2/91). Groups of 30 rats/sex were administered Picloram (AGR 274601, 80.3% purity) in the diets at 0, 20, 200, or 1000 mg/kg/day, 7 days/week for two generations. No treatment-related effects in clinical signs, body weights, and food consumption were reported. F0 and F1 adult males from the high dose group exhibited increased relative kidney weights, increased incidences of blood in urine and renal tubular and papillary degeneration/ regeneration and inflammation, ranging from focal to multifocal, unilateral or bilateral. Similar, but less frequent and/or less severe gross and histopathological alterations of the kidneys were observed in F0 and F1 females exposed to 1000 mg/kg/day. No treatment-related changes in fertility indices, gestation length, time to mating, litter size or gross - and histopathology of the reproductive organs were detected. In addition, neonatal survival, pup weight, and sex ratio were not affected. Parental NOEL (M/F) = 200 mg/kg/day (based on abnormal histopathological changes in the kidney). Reproductive NOEL = 1000 mg/kg/day (based no effects at HDT). **Acceptable**. (Leung, 8/20/98).

292-013; 36438; "Results of Fertility and Reproduction Studies in Rats Maintained on Diets Containing Tordon Herbicide (Picloram)."(D.D. McCollister et. al., Biochemical Research Laboratory, Dow Chemical Co., Midland, MI, 1/24/67). Picloram (approx. 95%) was administered in the diet at 0.03%, 0.1% or 0.3% (no record of total consumption) for a 3 generation, 2 litter reproduction study. 4

males and 12 females per group; exposed only 4 weeks prior to first mating; **no adverse effects indicated** on reproduction reported; **unacceptable** (insufficient number of animals, only 4 weeks of exposure prior to first mating, mating in groups, inadequate number necropsied, lack of histopathological exam in parental rats, inadequate dose level justification, and data not clearly presented), **not upgradeable**. (Gee, 11-21-85; updated, Leung, 7/28/98).
EPA one-liner: NOEL=1000 ppm, LEL=3000 ppm, Supplementary (10/26/84).

TERATOLOGY, RAT

** 52424-026 166807 "A Teratogenicity Study in Rats with Picloram K Salt" (Schroeder, R. 833-Bio/dynamics, Inc. East Millstone, New Jersey, Project # 89-3459, 1/23/90). Potassium (K) Salt of Picloram, aqueous solution (lot AGR 276452, purity of 34.7%) was administered via oral gavage (dissolved in distilled water) to 30 mated CD ^{7} female rats/dose at levels of 0, 100, 500 or 1000 mg/kg (corrected for purity), on gestation days 6-15 with cesarean sectioning on day 20 of gestation. Clinical signs in 1000 mg/kg dams included increased salivation. There were no significant reductions in mean maternal body weights, weight gain or food consumption. No adverse effect of treatment was evident from maternal gross postmortem evaluation. **Maternal NOEL= 500 mg/kg/day** (based on increased salivation at 1000 mg/kg). Litter data showed no apparent differences in the number of corpora lutea, implants, resorptions, or sex ratio. There were no compound-related effects on fetal body weights or the incidence of fetal malformations or variations. **No Adverse Effects. Developmental NOEL= 1000 mg/kg** (based on a lack of dose-related effects up to the high dose level). **Acceptable**. Kellner, 5/28/99.

292-013, 021; 36448, 52074; "Results of Teratogenic and Postnatal Studies in Rats Treated Orally with 4-amino-3,5,6-trichloropicolinic acid (Picloram)." (D.J. Thompson et. al., Dept. Of pathology & Toxicology, Human health Research & Development Lab., Dow Chemical Co., Zionsville, IN, Journal article (1972) and Dow report, (11/16/70). Picloram (no purity stated, lot 2RS17) in corn oil at 0, 500, 750, or 1000 mg/kg administered by oral gavage on days 6-15 of gestation (Day 0=+vag. smear) to Sprague Dawley rats (35/group: 25 for teratology study and 10 for postnatal study); 18-25 litters/group at Day 20 necropsy; 6-10 litters/group from natural delivery. Initial review (Gee, 11/22/85) indicated mortality at 750 and 1000 mg/kg (5/25 and 7/25, respectively; occurring between days 7 and 15 of gestation). Clinical signs in mid and high dose rats included mild diarrhea and hyperesthesia following 1 to 4 days of treatment, and severe ataxia and tremors in one rat from 1000 mg/kg following third dose. maternal NOEL (systemic)= 500 mg/kg(based on mortality and clinical signs). Subsequent review (Harnois, 9/25/87) of additional data (292-021) noted delay in ossification (all levels, 5th sternebra); hydronephrosis, extra ribs (1000 mg/kg); hydroureter (750 mg/kg); no excess death, delay in maturation, or remarkable necropsy findings (weanlings, 2/sex/group) in delivered pups reported; apparent developmental NOEL = 500 mg/kg. **No adverse effect** as ossification effect reported as transient and other effects occurred with maternal toxicity. **Unacceptable, not upgradeable** (no purity of test article, absence of dosing solution analysis, inadequate maternal weight data for determination of dose, missing necropsy sheets, inadequate numbers of weanlings necropsied, unclear description of test conduct, inadequate clinical observations on dams, questionable maternal health, missing historical control data for ossification defects or kidney-related findings). (Updated, Leung, 7/29/98).

EPA one-liner: Supplemental. Teratology NOEL > 1000 mg/kg (HDT); Fetotoxic NOEL = not determined.

TERATOLOGY, RABBIT

** 292-012, 021, 023 and 52424-016, 038; 17794, 52075, 56946, 162065, 170553, respectively; "Picloram Potassium Salt: Oral Teratology Study in Rabbits." (J.A. John et. al., Toxicology Research Lab., Health & Environmental Sciences, USA, Dow Chemical USA, Midland, MI, 1/17/84). Potassium salt of Picloram (AGR 204028, 37.3% acid equivalency) in water at 0, 40, 200 or 400 mg/kg was

administered by oral gavage to artificially inseminated (Day 0) New Zealand White rabbits (25/group) on Days 6-18; 18-23 litters/group examined; analysis of dosing solution indicated 96-114% of nominal. Initial review (Gee, 7-25-85) noted doubling in resorptions at 400 mg/kg and maternal NOEL (transient decreased body weight gain) = 40 mg/kg; insufficient information for evaluation of developmental effect; unacceptable, upgradeable (no individual data). Subsequent review of individual data (023 56946 and 021 52075), registrant's comments (021) found dose-related increase in resorptions. **No adverse effects indicated**: omphalocoele at all levels and persistent truncus arteriosus at 200 mg/kg were not considered to be treatment-related because the incidences of these malformations were within historical control values (52424-016, 162065). 2 fetuses in 2 litters with hemivertebra at 400 mg/kg was also seen in concurrent controls and was not considered to be treatment-related. Developmental NOEL = 400 mg/kg. Study previously unacceptable (data were still needed to verify the amount of test material or dosage administered), concentration of active ingredient in dosing preparations verified (52424-038, 170553). **Study acceptable.** (Harnois, 8/25/87; updated and revised, Leung, 7/29/98; 6/8/99, rerevised, Moore, 10/13/99).

GENE MUTATION

292-013; 36439; "Evaluation of Herbicides for Possible Mutagenic Properties: Point Mutations in Salmonella typhimurium With Picloram." (K.J. Andersen et. al., Columbus Laboratories, Battelle Memorial Institute, Columbus OH, Journal article published in J. Agr. Food Chem. 20: 649, 1972). 110 herbicides tested for ability to induce point mutations in Salmonella typhimurium; picloram was negative with 8 strains (not identified); T_4 phage strains were used with E. coli B and KB as indicators, picloram at 500 ug per (20 mls ??) with rII T_4 or 6000 ug (per ??) with AP72 T_4 plated with B or KB and difference in number of plaques evaluated; **unacceptable**. (Gee, 11/21/85; updated, Leung, 7/30/98).

292-013; 36441; "Mutagenic and Recombinogenic Action of Pesticides in Aspergillus nidulans: Point Mutations." (M. Bignami et. al., Istituto Superiore di Sanita, Rome, Italy, Journal article published in Mutation Res. 46: 395-402, 1977). 13 pesticides, including picloram (purity not provided) tested for point mutations to 8-azaguanine resistance (strain 35), mitotic crossing-over and mitotic non-disjunction and haploidization (strain P) in Aspergillus nidulans; **no adverse effects reported; unacceptable**. (Gee, 11/21/85; updated, Leung, 7/30/98).

292-013; 36443; "Microbiological Mutagenicity Studies of Pesticides in vitro : Induction of Point Mutations in Salmonella typhimurium." A. Carere et. al., Istituto Superiore di Sanita, Roma, Italy, Journal article in Mutation Res. 57: 277-286, 1978). Picloram (99.9% purity) one of 14 pesticides tested for induction of mutations in 4 strains (TA 1535, 1536, 1537 and 1538) of Salmonella typhimurium in the spot test +/- S9 (rat liver with phenobarbital activation); no increase in mutation rate reported; **Unacceptable**. (Gee, 11/21/85; updated, Leung, 7/30/98).

292-013; 36444; "Microbiological Mutagenicity Studies of Pesticides in vitro: Induction of Resistance to Low Concentrations of Streptomycin in Streptomyces coelicolor." (A. Carere et. al., Istituto Superiore di Sanita, Rome, Italy, Journal article in Mutation Res. 57: 277-286, 1978). Picloram (99.9% purity) tested in "disk" assay at 200 ug on Streptomyces coelicolor for resistance to streptomycin; spontaneous = 6, induced = 244; **Possible adverse effect indicated**: positive forward mutation from sensitivity to resistance; **Unacceptable**. (Gee, 11/21/85; updated, Leung, 7/30/98).

** 292-021; 52078-79; "Salmonella mutagenicity tests: II. Results from testing of 270 chemicals" (K. Mortelmans et. al., Case Western Univ., Cleveland, OH; SRI International, Menlo Park, CA; and Microbiological Associates, Bethesda, MD, Journal article published Method in publication in Environmental Mutagenesis v8, suppl. 7 1-119, 1986;). Picloram (>97% purity) was tested in Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 at 0 (DMSO), 100, 33.3, 1000, 3333.3 and 10000 ug/plate with or without S9 activation (derived from liver homogenates prepared from Aroclor-1254-treated male Sprague-Dawley rats and male Syrian Hamsters). Triplicate plates/dose level and all assays were repeated. Test material was preincubated with one of the test strains for 20 minutes at 37°C

prior to addition of soft agar and plating on minimum agar plates for detection of induced mutants. Positive controls were functional. No increase in revertants in any strain with or without S9 metabolic activation. **No adverse effects indicated; acceptable**. (Harnois, 8/13/87; updated, Leung, 7/30/98).

292-021; 52080, 60272; "Chemical mutagenesis testing in Drosophila--results of 53 coded compounds tested for the National Toxicology Program" (R.C. Woodruff et. al., Dept. Of Biological Sciences, Bowling Green State Univ., Bowling Green, OH; Dept. Of Zoology, Univ. Of Wisconsin, Madison, WI; Div. Of Biology and Medical Sciences, Brown Univ., Providence, RI, journal article published in Environ. Mutagenesis 7: 677-702, 1985) Picloram (no purity or composition) was administered to adults by feeding and injection for testing in the sex-linked recessive lethal and reciprocal translocation assays. **No adverse effect reported; unacceptable** (summary data only, no purity analysis), **but possibly upgradeable**. (Harnois, 8/18/87; updated, Leung, 7/30/98).

52424-016; 162063 (formerly 292-022; 54351) "Evaluation of Picloram in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay." (V. A. Linscombe and B. Bhaskar Gollapudi, Lake Jackson Research Center, Dow Chemical Co., 1-87) Picloram (lot no. AGR 219562, 93.4%purity) in DMSO was tested in a forward mutation assay at 125, 250, 500, 625 or 750 ug/ml without S-9, at 250, 500, 750, 1000 or 1250 ug/ml with S-9 mix (derived from liver homogenates prepared from Aroclor-1254-treated (500 mg/kg) male Sprague-Dawley rats). CHO-K_1BH_4 cells were exposed to the test article for 4 hours at 37°C. Single trial with 5 plates. Positive controls functional; Picloram did not induce a significant increase in the frequencies of TG-resistant mutations in the CHO-K_1BH_4 cells**. No adverse effects indicated. Unacceptable and not upgradeable** (no repeat test). (Harnois, 8/7/87; updated, Leung, 7/29/98).

** 52424-004; 161900; "Evaluation of Picloram in the Ames Salmonella/Mammalian-Microsome Bacterial Mutagenicity Assay"; (Y.E. Samson and B. Bhaskar Gollapudi, Health & Environmental Sciences - Texas, Lake Jackson Research Center, Dow Chemical Co., Freeport, TX, Study ID # K-038323-046, 4/27/90). *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were treated for 48 hours at 37°C with picloram (AGR 274601, 80.3% a.i.) At concentrations from 5 to 5000 ug/plate with and without metabolic activation. Two separate trials were performed with triplicate plates for each dose. S9 fraction was prepared form male Sprague-Dawley rats treated with Aroclor 1254 (500 mg/kg) 5 days prior to collection of livers. Positive controls functional. No adverse effects. No treatment-related increase in the incidence of reverse mutation. **Acceptable**. (Leung, 8/5/98).

Summary:
Newly submitted (negative) findings (from Salmonella, Drosophila, mammalian cells) close this data gap and indicate that the evaluation of "adverse effects" for this category in the previous summary may be mitigated. These test systems are widely used, well-validated and useful in evaluation of potential adverse effects in mammals. Although the test with Streptomyces coelicolor has interesting data and appears to have been well-conducted, the positive results have not been confirmed in other laboratories. These positive findings are noted in the current summary, but the results from the more widely validated tests, although negative, are considered to have greater relevance for the identification and regulation of potential hazards. The overall evaluation for this category of tests is that no adverse findings have been demonstrated in validated assays.

CHROMOSOME EFFECTS

52424-016; 162064 (formerly 292-013 36449) "Cytogenetic Effects of Picloram on the Bone Marrow Cells of Rats." (D.C. Mensik et. al., & Environmental Research, Dow Chemical Co., Freeport, TX, 12/29/76) . Picloram (AGR 145492, 89% purity) given by gavage to rats at 0, 20, 200, or 2000 mg/Kg in single dose; 5/sex/group; Approximately 21 hours after dosing, all animals were treated intraperitoneally with 1.0 mg/kg of colchicine. Three hours later, bone marrow cells were collected. No increase in aberrations were reported. **Unacceptable** (single sampling time, no concurrent positive control, no individual data, no info on body weights or mitotic index, no justification of dose other than LD50 is 3750

to 8200 mg/kg), **not upgradeable**. (Gee, 11/21/85; updated, Leung, 7/29/98).

EPA one-liner: minimum and negative to 2000 mg/Kg (10/26/84).

292-021; 60271; "NTP-Unpublished results--in vitro cytogenetics protocol summary, chromosome aberration test" (Litton Bionetics, 1983). Picloram (48749; no purity) in DMSO at 800-2500 ug/ml in CHO cells with activation and without activation; harvest at 22.5 hrs. **Adverse effects indicated:** (increase in aberrations with and without activation, all levels). Although the findings are well-presented, the study is **unacceptable** because of an incomplete description of the test procedures. (Harnois, 8/24/87; updated, Leung, 7/31/98).

** 292-013, -021 and 52424-028,-038; 36445, 52076, 166810, 170554, respectively; "Evaluation of Picloram in the Mouse Bone Marrow Micronucleus Test." (B. Bhaskar Gollapudi et. al., Lake Jackson Research Center, Dow Chemical Co., Freeport, TX, 4/85). Picloram (92%, lot AGR 177446) in 0.5% methocel was administered by gavage at 0, 171, 514 or 1543 mg/Kg in single dose to CD-1 (ICR) BR mice for micronucleus assay. 5/sex/group; sac at 24 and 48 hrs; marrow sample taken the day after sac; 1000 polychromatic erythrocytes/animal were scored for micronucleated polychromatic erythrocytes. **no adverse effect reported;** Unacceptable (no individual data, no justification for delay in marrow sampling, only 2 sampling times), not upgradeable (Gee, 11/21/85). Review of individual data (021 52076) confirms summary of data, but status remains unchanged (only 2 sampling times) (Harnois, 8/10/87; updated, Leung, 7/30/98). Study was deemed unacceptable because bone marrow samples were obtained or harvested from both femurs on the following day after sacrifice; information provided in 52424-038, 170554 noted that the text of the report was in error, the bone marrow samples were collected “in the following way” and not “on the following day”. **Acceptable.** (Leung, 6/8/99, revised, Moore, 10/14/99).

Summary:
This test shows positive findings *in vitro* both with and without activation. Data from *in vivo* assays using bone marrow as the source of cells are negative. Comparisons of *in vivo* and *in vitro* test results are difficult. In the *in vitro* study, the cells are directly exposed to the substance and effects can be evaluated. In the *in vivo* study, there is always doubt that the bone marrow is a target organ for an orally administered substance.

DNA DAMAGE

292-021; 59148; "Biological mechanisms involved in the toxicity of Picloram herbicide in rats" (T.R. Fox et. al., Toxicology Research Lab., Health & Environmental Sciences, USA, Dow Chemical USA, Midland, MI, 8/84). ^{14}C-Picloram (potassium salt, 98.8% radiochemical purity, specific activity 10.7 mCi/mmole) was administered by gavage at 1063 mg/kg (about 370 uCi/animal) to 4 male rats with 2 rats as negative controls; after 4 hours, livers removed and pooled into a treated group and an untreated group; DNA purified and repurified; **No adverse effect** - no differences were seen between negative control and two experimental liquid scintillation counts of radioactivity; **Supplementary study** to investigate the mechanism of oncogenicity. (Davis 12/1/87; updated, Leung, 7/31/98).

292-021; 52077; "Biological mechanisms involved in the toxicity of Picloram herbicide in rats" (T.R. Fox et. al., Toxicology Research Lab., Health & Environmental Sciences, USA, Dow Chemical USA,

Midland, MI, 8/84). Picloram (free acid, no purity) was tested at 5 concentrations (5×10^{-4} to 5×10^{-6} molar) in primary cultures of rat liver hepatocytes (Williams assay). Liver cells were incubated with picloram and ^{3}H-thymidine for 18 hours at 37°C. DNA repair was quantitated by evaluating the incorporation of ^{3}H-thymidine into nuclear DNA by microradiography. **No adverse effect reported** for 30 cells/treatment; **Supplementary study** to investigate the mechanism of oncogenicity. (Davis 12/1/87; updated, Leung, 7/31/98).

292-021; 60270 "NTP-Unpublished Results- in vitro cytogenetics protocol and summary, SCE test"; (Litton Bionetics, 1983) Picloram (48749; no purity) in DMSO at 25-833 (without activation) and 250-2500 ug/ml (with activation) in CHO cells for 26 hrs; BUDR added after 2 hrs; harvest at 28 hrs. **Adverse effects indicated**: (increase in SCE's starting at 250-500 ug/ml with and without activation). Although the findings are well-presented, the study is **unacceptable** because of an inadequate description of the test. Upgradeable. (Harnois, 8/24/87; updated; Leung, 7/31/98).

** 52424-016; 162062; "Evaluation of Picloram in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay" (M. L. McClintock and B. Bhaskar Gollapudi, Health & Environmental Sciences-Texas, Lake Jackson Research Center, Dow Chemical Co., Freeport, TX, Study ID # K-038323-047, 6/21/90). Primary rat hepatocyte cultures were exposed to picloram (AGR 274601, 80.3% a.i.) at concentrations ranging from 10 to 1000 ug/ml for 18 to 20 hours at 37 °C. Vehicle (DMSO: 1%) and positive controls (2-AAF: 2.233 ug/ml) were included in assay. Quadruplicate cultures/ dose level were conducted in each of two separate trials. Positive controls were functional. **No adverse effects:** Test material did not elicit a positive UDS response in rat hepatocyte cultures. **Acceptable** (Leung, 8/7/98).

OTHER MECHANISMS

292-013; 36446; "Analysis of Mitotic Nondisjunction with Aspergillus nidulans." (G. Morpurgo et. al., Istituto dell=Orto Botanico and Istituto Superiore di Sanita, Rome, Italy, Journal article published in Environmental Health Perspectives, 31: 81-95, August, 1979). Picloram (purity not provided) tested at maximum level of 0.8 mg/ml for induction of nondisjunction with a diploid strain of Aspergillus nidulans; **no adverse effect indicated; unacceptable**. (Gee, 11/21/85; updated, Leung, 7/30/98).

292-021; 59149; "Biological mechanisms involved in the toxicity of Picloram herbicide in rats" (T.R. Fox et. al., Toxicology Research Lab., Health & Environmental Sciences, USA, Dow Chemical USA, Midland, MI, 8/84). Picloram (potassium salt, 97.5% purity) was administered in one dose by gavage at 1063 mg/kg to 6 male F344 rats with 6 rats as negative controls; 48 hours later ^{3}H-thymidine was injected and 4 hours after that the rats were sacrificed; 9 doses of 847 mg/kg/day were given to 6 rats (6 additional rats as negative controls) with ^{3}H-thymidine injection 24 hours after the last dose and sacrifice 4 hours after that; liver DNA was purified from all rats with specific radioactivities determined in all cases and autoradiography done only for the single dose animals; mice served as controls for the induction of cell division and DNA repair synthesis; **No adverse effect** - no differences were seen between negative control and two experimental cell regeneration frequencies. **Supplementary study** to investigate the mechanism of oncogenicity. (Davis 12/1/87; updated, Leung, 7/31/98).

Summary:
Because of the possibility that orally administered test substances may not reach bone marrow *in vivo*, other organs are sometimes sampled for genetic effect. The liver tests, although inadequate and in this instance, not specified as part of the genotoxic test battery, are a commendable attempt to increase observations in whole animals. The *in vitro* UDS assay is acceptable. However, the SCE assay was noted as unacceptable but possibly upgradeable. The SCE assay appears to have been well-conducted but requires a more complete test description before being accepted. This test produced positive findings. Overall - there is no data gap in this category.

NEUROTOXICITY

No study on file.

SUBCHRONIC STUDIES

(90-day feeding study)

52424-005; 161901; "Results of a Six-Month Dietary Toxicity Study of Picloram (4-amino-3,5,6-Trichloropicolinic Acid) Administered in the Diet to Male and Female Beagle Dogs"; (T. Barna-Lloyd et. Al., Health & Environmental Sciences, Dow Chemical USA, Midland, MI, 6/9/82). Picloram technical (Lot # AGR-177446, 91.2% purity) was administered to 6 beagle dogs/sex/dose at 0, 7, 35 or 175 mg/kg/day for 6 months. High dose animals exhibited reduced body weight gain with decreased food consumption. Increased absolute liver weight was reported in high dose males and females and mid dose males without any treatment-related changes in histopathology. **No adverse effects indicated**. NOEL (M/F) = 7 mg/kg/day (based on liver weight changes). **Unacceptable and not upgradeable** (lack of ophthalmological examination). (Gee, 7/85; updated and revised, Leung, 7/28/98).

52424-005; 161902; "Technical Grade Picloram: Results of a 13-Week Dietary Toxicity Study in Fischer 344 Rats"; (S.J. Gorzinski et. al., Health & Environmental Sciences, Dow Chemical Co., Midland, MI, Study ID # K-038323-(32), 7/1/82). Fischer 344 rats (15/sex/dose) were fed diets to provide 0, 15, 50, 150, 300, or 500 mg/kg/day of picloram (technical grade, AGR 177446, 92% purity) for 13 weeks. All rats survived until scheduled sacrifice. No treatment-related changes in behavior or physical appearance were reported. Males and females exhibited significant increased relative liver weights at the highest three doses. Histopathological examination revealed slight enlargement and decreased staining affinity (pallor) of the centrilobular hepatocytes. These effects were observed in males fed 150 (3/10), 300 (7/10), or 500 (9/10) mg/kg/day. Similar effects were also noted in females treated at 150 (1/10), 300 (8/10), or 500 (10/10) mg/kg/day. **No adverse effects indicated**. NOEL (M/F) = 50 mg/kg/day (based on histopathological changes in liver). Study **unacceptable and not upgradeable** due to lack of ophthalmology. (Leung, 8/11/98).

(Dermal)

52424-025; 166806; "Picloram Potassium Salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits"; (Atkin, L. et al, The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI, Laboratory Project Study ID K-050731-008, 1/31/90). 822. Aqueous Solution of Picloram-K^+ (Tordon K^+ Salt Liquor) (no lot number, 35.2% a.i. (30.4% acid equivalent)) was applied to the shaved back of 5 New Zealand White rabbits per sex per dose level at doses of 0 (distilled water), 75.3 (diluted in distilled water), 251 (diluted in distilled water), or 753 (undiluted) mg/kg/day for 6 hours per day for a 21 day interval (15 applications, weekends excluded) using a dressing and elastic jacket. No animals died. No treatment-related clinical signs were observed. Treatment-related very slight (grade 1) erythema and edema at 75.3 mg/kg/day, very slight erythema and very slight to well-defined (grade 2) edema at 251 mg/kg/day, and very slight to well-defined erythema and edema at 753 mg/kg/day were observed at the test site during the study. Clinical chemistry revealed no treatment-related effects. Macroscopic and microscopic examinations of the internal organs revealed no treatment-related abnormalities. Macroscopic examination of the treated skin revealed treatment-related slightly thickened skin in males and focal hyperemia in females at 753 mg/kg/day. Microscopic examination of the treated skin revealed treatment-related very slight multifocal epidermal hyperplasia in both males and females at all dose levels. **No adverse effects.** NOEL (systemic, M/F)=753 mg/kg/day (based on no treatment-related effects at HDT), NOEL (dermal, M/F)< 75.3 mg/kg/day (based on a treatment-related skin irritation and very slight multifocal epidermal hyperplasia). **Supplemental** (formulation rather than technical grade a.i. used). (Corlett, 6/7/99)

METABOLISM STUDIES

52424-027; 166809; "Picloram: General Metabolism Studies in Female Fischer 344 Rats"; (R. H. Reitz, et. Al., Toxicology Research Lab., Health & Environmental Sciences, Dow Chemical Co., Midland, MI, Lab. Project Study IF # K-038323, 8/10/89). Four groups containing 5 female Fischer 344 rats/group received single oral dose of ^{14}C-Picloram (Lot # GHD 1265-40A, 16.28 mCi/mmole, >99.5% radiochemical purity) and unlabeled Picloram (AGR #221371, 99.4% purity) at 10 or 1000 mg/kg. A third group were pretreated with 14 daily oral doses of unlabeled Picloram at 10 mg/kg

followed by a single oral dose of ^{14}C-Picloram on day 15. Fourth group was given ^{14}C-Picloram as a single IV dose at 10 mg/kg. Picloram is primarily excreted unchanged in the urine (69 to 86%) and feces (5 to 25%). Mean percent of dose present in selected tissues, carcass , and expired CO_2 was not detectable at 72 hours. Although disposition of radioactivity was similar in all groups, significantly lower amounts of radioactivity were recovered in the feces after iv dosing. There were no significant changes in the rates or routes of disposition of ^{14}C-Picloram after administration of a series of 14 daily doses of 10 mg/kg. Clearance of radioactivity from the plasma is well described by a two compartment model. The terminal rate of clearance of ^{14}C from the plasma at a dose of 10 mg/kg is apparently first order with a half-life of approximately 88 minutes, and the initial rapid phase had a half-life of 5.0 minutes. **Supplemental** (Only female rats were employed; Leung, 6/1/99).

52424-027; 166808; "Kinetics of ^{14}C-Labeled Picloram in Male Fischer 344 Rats"; (R. J. Nolan et. al., Toxicology Research Lab., Health & Environmental Sciences, Dow Chemical Co., Midland, MI, 2/4/80). Groups of 3 male Fischer 344 rats received single doses of ^{14}C-labeled picloram (Lot # 188; GH-14-55A, 10 mCi/mmol, >99% radiochemical purity) and unlabeled picloram (Lot # AGR155434, technical grade) intravenously (14 or 160 mg/kg) or orally (9.6 or 1634 mg/kg). Picloram was rapidly eliminated primarily in the urine by a saturable process and was not sequestered in major tissues or organs of the rat. Over 84% of both intravenous and oral doses were recovered in the urine and a minor fraction of the dose (2.1 to 10.7%) was eliminated via the feces. 0.42 and 0.04% of the dose remained in the tissues and carcass following 48 hours after iv administration of 14 and 160 mg/kg, respectively. However, slightly higher fraction of the administered dose (9.6 mg/kg or 1634 mg/kg) was found in tissues and carcass (0.16% and 6.48%, respectively). The tissue distribution of radioactivity following the 9.6 mg/kg oral dose was consistent with the intravenous data in that the GI tract plus contents was the only tissue in which detectable amount of radioactivity were found. In contrast, detectable amounts of radioactivity were found in all tissues obtained from rats treated at 1634 mg/kg. Most of the radioactivity in tissues from rats treated at 1634 mg/kg was found in the GI tract plus contents and probably represents unabsorbed picloram which would be excreted in the feces. Concentration of radioactivity in the plasma following iv dosing is consistent with Michaelis-Menten (saturation) kinetics. The disappearance of radioactivity from the plasma following 14 mg/kg dose was biphasic with no evidence of saturation with half lives for the rapid initial and slow terminal phases of 6 and 100 minutes, respectively. In contrast, the disappearance of radioactivity from plasma following 160 mg/kg exhibited saturation kinetics. During the first 1.5 hour after dosing, the rate at which radioactivity disappeared form the plasma increased until it equaled the rate observed following the 14 mg/kg dose. Following oral administration, the concentration of ^{14}C-picloram in the plasma peaked within 5 minutes but Cmaxs were not proportional the to dose administered. Five minutes after dosing with 9.6 and 1634 mg/kg, the plasma contained 4.37 and 193 ug ^{14}C-picloram/g, respectively. Thus a 170 fold increase in the dose resulted in only a 44 fold increase in Cmax. Following dosing with 1634 mg/kg orally, plasma concentration of ^{14}C-picloram was constant for 4 hours and then decreased in a biphasic manner with an apparent terminal half life of 15.2 hours. **Supplemental** (Leung, 6/2/99).